

**Claims**

5 1. A method of determining voltage changes by means of a voltage-sensitive dye, characterized in that the voltage-sensitive dye is irradiated with light having a wavelength at which the dye has an absorption  $\leq 20\%$  of its absorption maximum and the fluorescence caused by irradiation with light is measured.

10 2. The method according to claim 1, characterized in that the wavelength of the irradiated light is such that the dye has an absorption of  $\leq 12\%$ , in particular  $\leq 8\%$  and preferably  $\leq 2\%$  of its absorption maximum at said wavelength.

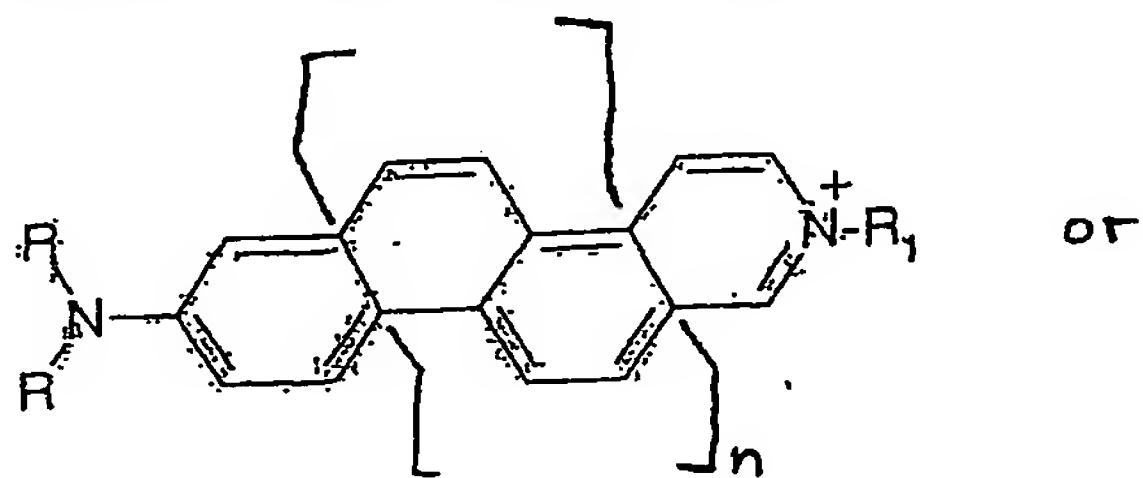
15 3. The method according to claim 1, characterized in that the wavelength of the irradiated light is in the longer wavelength range, related to the absorption maximum.

20 4. The method according to any of the preceding claims, characterized in that an increase or decrease of fluorescence is measured.

5. The method according to any of the preceding claims, characterized in that it is used to determine voltage changes in cells.

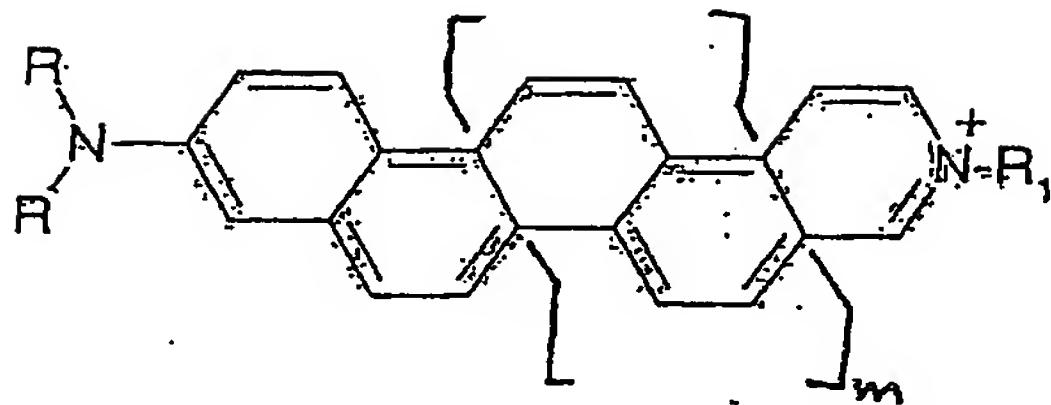
25 6. The method according to any of the preceding claims, characterized in that it is used to determine voltage changes in membranes, especially cell membranes.

30 7. The method according to any of the preceding claims, characterized in that, as voltage-sensitive dye, a compound of formula (I)



or formula (II)

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is used, wherein

each R independently is a hydrocarbon residue, which optionally can be substituted with hydroxyl,

R<sup>1</sup> is a monovalent residue,

n is an integer from 1 to 9, and

n is an integer from 0 to 8,

which compounds optionally can have one or more substituents at ring carbon atoms.

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8. The method according to any of the preceding claims, characterized in that ANNINE-4, ANNINE-5, ANNINE-6, ANNINE-7, ANNINE-8 or ANNINE-9 is used as a voltage-sensitive dye.

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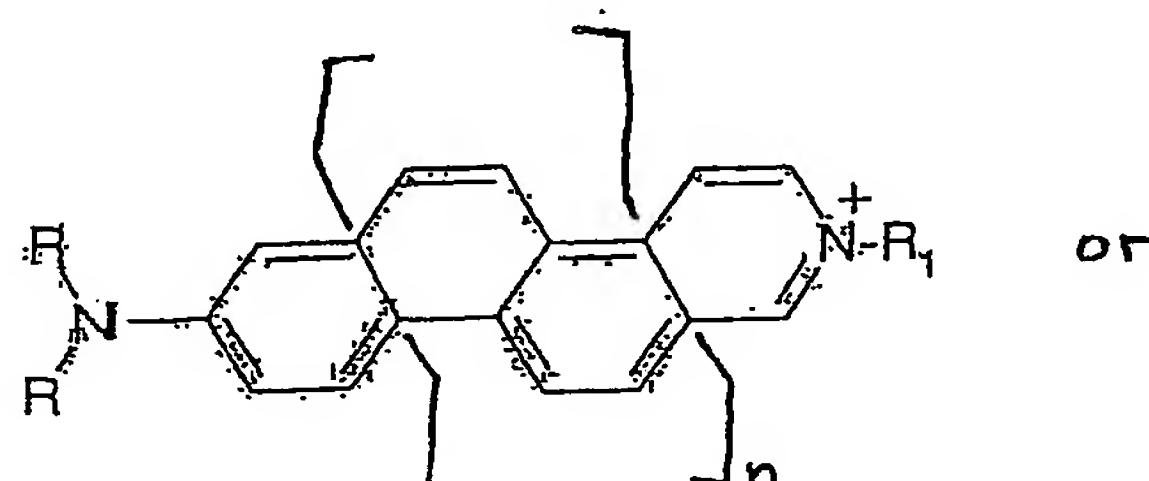
9. The method according to any of the preceding claims, characterized in that a change of fluorescence radiation caused by the Stark effect is measured.

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10. The method according to any of the preceding claims, characterized in that a two-photon excitation is effected.

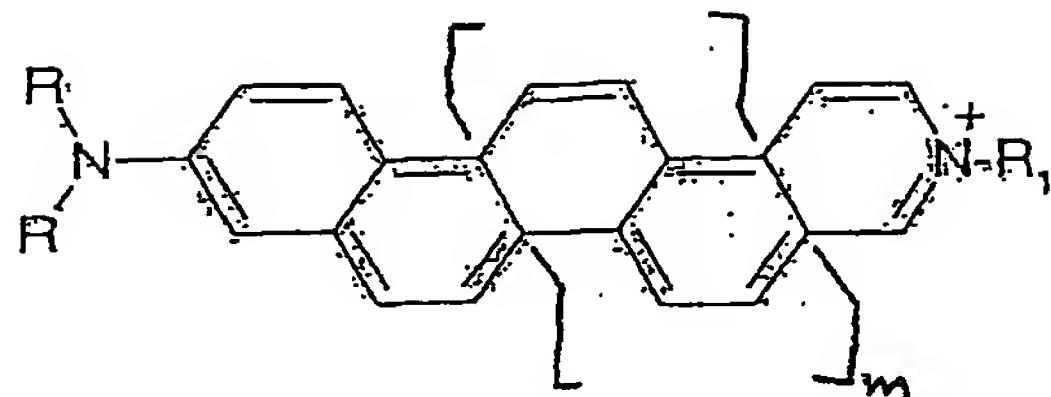
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11. Voltage-sensitive dye having the formula (I)



or formula (II)

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wherein

each R independently is a hydrocarbon residue, which optionally can be substituted with hydroxyl,

10 R<sup>1</sup> is a monovalent residue,

n is an integer from 1 to 9, and

n is an integer from 0 to 8,

which compounds optionally can have one or more substituents at ring 15 carbon atoms.

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